PB86-129814

Preneoplastic Transformation of Respiratory Epithelial Cells by Complex Organic Mixtures in a Clonal Assay

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

Nov 85

U.S. Department of Commerce
National Technical Information Service
NTIS.

PB86-129814

EPA/600/D-85/283
November 1985

PRENEOPLASTIC TRANSFORMATION OF RESPIRATORY EPITHELIAL CELLS BY COMPLEX ORGANIC MIXTURES IN A CLONAL ASSAY

by

Marc J. Mass
Respriatory Carcinogenesis Group
Carcinogenesis and Metabolism Branch
Health Effects Research Laboratory
Research Triangle Park, NC 27711

Clyde M. Marr, III
Environmental Health Research and Testing, Inc.
Research Triangle Park, NC 27709

Judy L. Mumford
Genetic Bioassay Branch
Health Effects Research Laboratory
Research Triangle Park, NC 27711

Health Effects Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA/600/D-85/283		PB86 123814/AS

4. TITLE AND SUBTITLE	5. REPORT DATE
PRENEOPLASTIC TRANSFORMATION OF RESPIRATORY EPITHELIAL CELLS BY COMPLEX ORGANIC MIXTURES IN A CLONAL ASSAY	November 1985
	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
M. J. Mass*, C. M. Marr, III,**, and J. L. Mumford*	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
*same as Block 12 **Environmental Health Research & Testing, Inc. Research Triangle Park, NC 27709	11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, NC 27711	14. SPONSORING AGENCY CODE EPA/600/11

15. SUPPLEMENTARY NOTES

16. ABSTRACT

In this study complex organic mixtures that were extracts of particulate emissions from 3 heating sources were tested for the presence of agents that induce preneoplastic transformation of rat tracheal epithelial (RTE) cells in an in vitro clonal assay. The samples were derived from homes in Xuan Wei County, China where extremely high incidences of lung cancer are found that are 4-fold in excess of the national average. In some communes, the cancer mortality rates are as high as 19 to 30 times the national average rate. This is postulated to result from inhalation exposures produced by the routine burning of a "smokey" coal in an open indoor pit as the sole source of heat and cooking fuel under unvented conditions. Dichloromethane extracts of particulate samples (<10 μm in size) of "smokey" coal obtained from the respirable air in homes burning this fuel were tested in the RTE cell transformation assay. In addition, the response was compared to that obtained from particulate emissions of 2 other fuel types used in communes in China that have low associated lung cancer incidences: communes that use "smokeless" coal, and wood as fuels. These samples were also analyzed by gas chromatography/mass spectrometry for polynuclear aromatic hydrocarbon (PAH) content. The organic extracts of smokey coal emissions produced significant increases in the number of transformed RTE cells exposed in vitro, in a reproducible manner. Extracts of smokeless coal exhaust and woodsmoke exhaust produced variable levels of transformation but were definitely positive in this assay. The difficulty in reproducibility is assumed to result from the insoluble nature of these complex organic mistures in the culture medium. The chemical analyses of selected PAH in the 2 types of coal were quite similar, but very different from woodsmoke exhaust extract. The results are discussed in relation to the level of particulate emissions, the profile of selected PAH, and the cancer incidences in the respective homes burning these fuels.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	29
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ABSTRACT

In this study complex organic mixtures that were extracts of particulate emissions from 3 heating sources were tested for the presence of agents that induce preneoplastic transformation of rat tracheal epithelial (RTE) cells in an *in vitro* clonal assay. The samples were derived from homes in Xuan Wei County, China where extremely high incidences of lung cancer are found that are 4-fold in excess of the national average. In some communes, the cancer mortality rates are as high as 19 to 30 times the national average rate. This is postulated to result from inhalation exposures produced by the routine burning of a "smokey" coal in an open indoor pit as the sole source of heat and cooking fuel under unvented conditions. Dichloromethane extracts of particulate samples (<10 μm in size) of "smokey" coal obtained from the respirable air in homes burning this fuel were tested in the RTE cell transformation assay. In addition, the response was compared to that obtained from particulate emissions of 2 other fuel types used in communes in China that have low associated lung cancer incidences: communes that use "smokeless" coal, and wood as fuels. These samples were also analyzed by gas chromatography/mass spectrometry for polynuclear aromatic hydrocarbon (PAH) content. The organic extracts of smokey coal emissions produced significant increases in the number of transformed RTE cells exposed *in vitro*, in a reproducible manner. Extracts of smokeless coal exhaust and woodsmoke exhaust produced variable levels of transformation but were definitely positive in this assay. The difficulty in reproducibility is assumed to result from the insoluble nature of these complex organic mixtures in the culture medium. The chemical analyses of selected PAH in the 2 types of coal were quite similar, but very different from woodsmoke

exhaust extract. The results are discussed in relation to the level of particulate emissions, the profile of selected PAH, and the cancer incidences in the respective homes burning these fuels.

INTRODUCTION

The National Cancer Survey of China for the years 1973-75, indicated that residents in Xuan Wei County have unusually high lung cancer mortality rates that are about 5 times the national average for China (Li et al., 1979). Xuan Wei is a rural county, located in Yunnan province in southern China where most residents in the county (93%) are farmers. The Xuan Wei women who rarely smoke have the highest lung cancer mortality rates among all counties surveyed, and the men's rates are among the highest. In view of this serious public health problem, Chinese and American investigators have jointly initiated multidisciplinary studies, including epidemiology, air monitoring, chemistry and toxicology to examine cancer etiology in Xuan Wei.

For generations, Xuan Wei County residents have burned coal or wood for domestic cooking and heating in a shallow unvented pit in the floor of the dwelling's main room. Women are traditionally responsible for maintaining the fire and for cooking meals, tasks that consume 4-6 h each day. The common fuels used in Xuan Wei County are "smokey" coal, "smokeless" coal and wood. The smokey coal contains more volatiles and smokeless coal contains a higher ash content. It has been postulated that the elevated lung cancer rates in Xuan Wei may be associated with domestic use of smokey coal. The communes with high lung cancer rates use smokey coal, whereas the communes that use wood and/or smokeless coal tend to have much lower lung cancer mortality rates.

Short-term in vitro bioassays with tracheobronchial tissues have shown that this epithelium responds to polynuclear aromatic hydrocarbons (PAH) (Lane and Miller, 1974) and particulate carcinogens (Mossman and Craighead,

1974) as well as to complex environmental organic mixtures (Schiff et al., 1981) by manifestation of hyperplasia, squamous metapasia, and occasionally cellular atypias in organ cultures. Cells from organ culture fragments contain mixed-function oxidases (Mass and Kaufman, 1978, 1983a) with high affinity for polynuclear hydrocarbon carcinogens and produce ultimate carcinogenic metabolites which bind to DNA (Mass and Kaufman, 1983b). Unfortunately, no direct evidence for malignant transformation as evidenced by bona fide invasive growth in the culture has yet been reported for organ cultures exposed to PAH carcinogens in vitro.

More recently, the ability to assess quantitatively the potency of pure carcinogens, inhibitors, and tumor promoters in a clonal cell transformation assay was demonstrated using rat tracheal epithelial (RTE) cells (Thomassen et al., 1983, Mass et al., 1984a, 1984b). These cells normally do not survive in culture for long periods of time and undergo senescence. However, when treated with carcinogens, RTE cells acquire the ability to survive culture conditions that cause normal cells to die. These colonies of "immortalized" RTE cells are easily quantified and are induced in a carcinogen concentration dependent fashion; their emergence follows one-hit kinetics (Thomassen et al., 1983, Barrett et al., 1983). These transformed cells can be passaged in culture, become anchorage-independent, and have been observed to form epidermoid carcinomas when injected into hosts (Steele et al., 1976). This epithelial cell transformation technique has a major advantage over fibroblast cell transformation systems in that the majority of fatal human cancers derive from epithelial cells of the tracheobronchial epithelium (Mass et al., 1985). The RTE cell assay is further distinguished because the target cells are initially biologically normal cells that are diploid. Changes in the ability of these cells to survive in culture or

changes in their growth characteristics may be considered a primary alteration due to carcinogen exposure, which is in contrast to some fibroblast cell transformation assays where the target cells are initially considered to be preneoplastic (Barrett et al., 1984). Previously, the RTE cell transformation assay has been used to study pure carcinogens of diverse classes as oncogenic transforming agents (Nettesheim and Barrett, 1984). The complex mixture cigarette smoke condensate also gave a positive response (Steele and Mass, 1985). As a further validation for this assay we chose to determine if the transforming agents present in environmentally-encountered heating sources used in Xuan Wei County, China could be detected in the RTE cell transformation assay.

METHODS

Complex Mixture Samples

Air sampling, sample preparations, and PAH analysis were conducted from one home in the Lai Bin commune (LB), which has a high cancer rate (about 22 times the national rate in China) and Re Shui commune (RS), which has a low cancer rate (similar to the national average rate in China). The fuels burned during sampling were those routinely used by most residents of the commune. Most (> 90%) of LB residents use smokey coal from local coal mines. Smokey coal was burned during sampling in a LB home. A case of lung cancer had occurred previously in this LB home that was sampled. For the RS commune, most residents use wood (62%) and the rest use smokeless coal. Air samples were collected from a home located in the RS commune burning wood. Due to logistical difficulties, the smokeless coal from the RS commune was burned in a home located in a central commune, Cheng Guan (CG), during sampling. The indoor air samples were collected by a high-volume sampler with 10 μm size-selective inlets which were used to collect particles mostly smaller than 10 μm on glass fiber filters at a flow rate of 1.2 m^3/min. Sampling was conducted during the periods of active fuel combustion for cooking averaging 4 h/day. Organic material was removed from the filters by Soxhlet extraction with dichloromethane for 16 h. Aliquots of the concentrated extracts were dried for determination of residue weight. Separate aliquots were solvent exchanged to dimethyl sulfoxide at a concentration of 4 or 20 mg/ml. The organic extracts were also quantitatively analyzed by gas chromatography/mass spectrometry using on column injection in the multiple ion detection mode (Nishioka et al., 1982). A Finnigan 4500 electric quadrupole mass spectrometer equipped with an INCOS 2300 data system and a Finnigan gas chromatograph was used.

RTE Cell Transformation Assay

RTE cells were isolated as previously described (Mass et al., 1984a, 1984b) from Fisher 344 specific pathogen-free male rats of 7 to 9 weeks in age (Charles River, Wilmington, DE) by protease digestion of the epithelium. Cells were collected, and then counted with an electronic particle counter (Coulter Electronics, Hialeah, FL), and seeded onto a monolayer of mouse 3T3 clone J2 cells in Ham's F-12 medium (Grand Island Biological Company, Grand Island, NY) containing 5% fetal bovine serum, 1 μM hydrocortisone, 1 μg/ml insulin, 100 units/ml penicillin, 100 μg/ml streptomycin. One day later the cells were exposed to the complex mixtures, or to the vehicle control DMSO. Exposures to the complex mixtures lasted 24 hours, after which, the dishes were washed with phosphate-buffered saline; the medium was then replaced. Each transformation experiment usually consisted of 30 35 mm culture dishes, of which 5 were stained after 1 week in order to calculate the cytotoxicity of the exposure by counting the remaining colonies. For the remaining dishes, after 7 days the 3T3 cell monolayer was removed by treatment of the culture dishes with 0.02% EDTA dissolved in phosphate-buffered saline. The removal of feeder cells serves to arrest the growth of normal cells whereas those transformed by the carcinogens present are able to continue growing in the absence of the 3T3 cell monolayer (Thomassen et al., 1983). In addition, at this time, the culture medium was changed to exclude pyruvate (Marchok et al., 1984), which also serves to select for carcinogen-altered cells. The cells were grown in the absence of pyruvate for 10 days, and then the complete medium was used for the remainder of the 42-day assay. To terminate the experiment the cells on dishes were fixed in absolute methanol and then stained with an aqueous solution of 10% Giemsa. Surviving transformed colonies were scored with the aid of a

dissecting microscope. Transformation frequencies per surviving cell were calculated by the P_0 method (Thomassen et al., 1983) from the Poisson distribution using $P_0=e^{-n}$. P_0 is the proportion of dishes with no colonies, n is the average number of transforming events per dish; n/CFC is the transformation frequency per colony-forming cell, where CFC is the number of colony-forming cells per dish.

RESULTS

Transformation of RTE Cells with Pure Carcinogens

Initially, studies were performed using the direct-acting methylating carcinogen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Frequencies of surviving, transformed epithelial colonies (foci) are produced in a concentration-dependent manner that is linear with respect to the logarithm of both concentration of MNNG and transformation frequency (Chart 1). The transformation frequency is corrected for cytotoxicity attributed to MNNG exposure by assessment of colony-forming efficiency (CFE).

Because the agents contained in the coal and wood exhaust extracts included PAH, it was necessary to verify that the RTE cell assay could detect PAH that required biotransformation for activity. In Table 1, the results of a concentration-response experiment with benzo(a)pyrene (BP) are shown. BP was not toxic to RTE cells over the concentration range tested, yet produced significant numbers of transformants in a concentration-dependent manner. The transformation response appears to reach a plateau at 10 μg/ml. This is not surprising since the K_m of BP for BP-monooxygenase is between 1 and 2 μM (0.25 and 0.5 μg/ml) in rat tracheal epithelial cell microsomes (Mass and Kaufman, 1983) and, therefore, 10 μg/ml is far above saturation for the monooxygenase.

Complex Mixtures

PAH Analysis

Table 2 is an analysis of some selected constituents of dichloromethane extracts of smokey and smokeless coal, and wood exhaust particles, as determined by gas chromatography/mass spectrometry. No nitropyrenes were detected. Three-ring PAH (anthracene, 1-methylphenanthrene) were more

prevalent in smokey coal, and were found at equivalent levels in smokeless coal and wood exhaust extracts. PAH containing 4-fused rings (1-methylbenzanthracene, and benzanthracene) were higher in concentration in both coal exhaust extracts than wood, as were the 5-fused ring PAH, benzopyrenes and cyclopenta(cd)pyrene. Smokey coal exhaust also contained a 10-fold higher concentration of the 6-fused ring PAH benzo(ghi)perylene than did either of the other 2 exhaust extracts. In general, wood exhaust extracts contained significantly less of the PAH constituents in these analyses on a per organic weight basis.

Toxicity of Complex Mixtures to RTE Cells

The extracts of smokey and smokeless coal were assessed for cytotoxicity in clonal assays for colony-forming efficiency of RTE cells (Chart 2). The agents were added to cell cultures one day after seeding of RTE cells and the cells were exposed to test compounds for 24 hr. Smokey coal exhaust extract LB(HAi)LKX elicited the greatest toxicity and killed 50% of RTE cells at approximately 40 μg/ml. The next most toxic exhaust extract was that from wood [HW(HWi)SSC]; the extract had a TD_{50} of about 50 μg/ml. The extract of exhaust from burning smokeless coal, CB(HBi)YTS, had a TD_{50} near 80 μg/ml. All the exhaust extracts produced cytotoxicity curves that are anomalous to those seen with pure agents tested previously in RTE cells. These cytotoxicity curves are characterized by sharp drops in cell survival after a particular threshold was reached. This effect is most pronounced in the cytotoxicity curve for CG(HBi)YTS where between 70 μg/ml and 90 μg/ml the survival of RTE cells varies between 100% and essentially no survival.

Transformation of RTE Cells by Complex Mixtures

Because the toxicity curves for the fuel exhaust extracts were characterized by precipitous decreases in survival in small increments of dose after a threshold, it was not feasible to perform concentration-response experiments for transformation. Secondly, we also knew that it was possible to transform RTE cells with BP at concentrations that elicited no detectable toxicity. Therefore, in order to evaluate these complex mixtures we chose the highest concentration on the concentration-response curve that elicited no toxicity as evidenced by CFE. It is notable, in fact, that the concentrations chosen actually appeared to enhance survival of RTE cells in many cases. Extracts of exhaust of sample LB(HAi)LKX, smokey coal, reproducibly transformed RTE cells at a concentration of 30 μg/ml in 3 experiments (Table 3). On average, about one-third of the culture dishes contained transformed colonies at this concentration. In a single experiment, sample CG(HAi)SSL was also used, which was derived from another home burning smokey coal and was different from sample LB(HAi)LKX . Both samples were equally as active in the transformation assay.

The smokeless coal exhaust extract CG(HBi)YTS elicited a positive response in transformation experiments that was extremely variable in magnitude (Table 4); in 5 experiments there was a 20-fold variation in transformation frequency; there was also some variation in toxicity attributed to exposure to this mixture, however, the amount of toxicity did not correlate with the transformation frequency.

Wood exhaust extract HW(HWi)SSC was tested at 30 μg/ml in 3 experiments (Table 5). Although there was little variation in toxicity of this extract to RTE cells, a 10-fold variation in transformation frequency was observed.

DISCUSSION

We have utilized the RTE cell transformation bioassay to investigate the presence of potential carcinogens in dichloromethane extracts of particulate emissions from burning of 2 types of coal and of wood. One of these fuel sources, "smokey coal" is under consideration as the etiologic agent responsible for a remarkably high incidence of lung cancer in a commune in China in which this coal is used as the primary energy source for cooking and heating. In the typical home in Xuan Wei County, coal or wood are burned in an open pit inside the living quarters without provisions for exhaust. In homes the level of particulates measured from burning smokey coal were as high as 30.4 mg/m^3, 1.8 mg/m^3 for smokeless coal, and 23.1 mg/m^3 for wood. The proposed levels for acceptable particulate concentration of particles <10 μm is 150 - 250 $\mu g/m^3$ for U.S. ambient air (Federal Register, 1984).

We found that exhaust from all 3 types of fuel produced RTE cell transformants, indicating the presence of potential carcinogens. Extracts of smokey coal exhaust produced rather consistent levels of transformation between experiments. However, smokeless coal and wood exhaust extracts produced significant but variable levels of transformation. The magnitude of the transformation frequency was similar in all 3 exhaust extracts, even though the concentrations used were somewhat higher for "smokeless coal". We attribute the variability in responses to the physical characteristics of these complex organic mixtures, as observed in another study (Lang et al., 1980). The mixtures were sparingly soluble in the culture medium and at high concentrations produced mixtures which were opalescent. Therefore,

precipitation of the mixtures on culture surfaces was unavoidable and, in addition, the constituents were not solvated. In that case, the full potential of these mixtures to transform RTE cells was probably not realized, and comparisons of potency of these complex mixtures based upon studies with RTE cells cannot be made with certainty. However, the results indeed demonstrate that respiratory epithelial cells in culture can be readily transformed by these complex mixtures.

In these experiments the RTE cells were supported by a 3T3 cell monolayer which may have enhanced metabolic activation of the complex mixtures. In previous studies it has been shown that RTE cells grown clonally without a feeder layer are transformed by benzo(a)pyrene (Steele and Mass, 1985) and by 7,12-dimethylbenzanthracene (Steele and Mass, unpublished) at levels equivalent to RTE cells grown with feeder layers, indicating that the RTE cells grown clonally maintain an adequate level of mixed-function oxidases or other activating system. In addition, metabolic capacity with respect to BP for rat tracheal epithelium has been demonstrated directly (Mass and Kaufman 1983a, 1983b, 1984, Cohen et al., 1979).

The results from these studies with RTE cells and fuel exhaust extracts do not allow assignment of the etiologic agent that is responsible for the elevated cancer incidence in Xuan Wei, since the cell transformation data, although unquestionably positive, is similar for all 3 samples. A more thorough evaluation of the risk posed by these fuel sources can be made if parameters relating actual exposure of the respiratory tract to these effluents are known. Presently, it is known that smokey coal emits a high level of particulates of respirable size, of which over 70% are organic. Smokeless coal emits far lower levels of particulates which are only about

30% organic. Wood emits paricles that are about 55% organic but the particulates from woodsmoke are much larger and are, therefore, less respirable than smokey coal (Mumford et al., in preparation). Therefore, the quantity of potential carcinogens emitted by these fuels per cubic meter of air is very different, as are the quantities of carcinogens that are deposited in the respiratory tract. These are likely contributing factors to the etiology of pulmonary cancer in Xuan Wei.

ACKNOWLEDGEMENT

The authors are grateful to the excellent assistance in air sampling from the staff at the Institute of Health in Beijing, the Hygiene and Anti-Epidemic Stations of Yunnan Province, Qu jing Region and Xuan Wei County, The Peoples' Republic of China. We thank Miss Joye C. Denning for skillful typing of this manuscript.

REFERENCES

Barrett, J. C., T. E. Gray, M. J. Mass, and D. G. Thomassen. 1983. A quantitative, clonal assay for carcinogen-induced alterations of respiratory epithelial cells in culture. pp. 325-340. In: Short-term Bioassays in the Analysis of Complex Environmental Mixtures III, M. D. Waters, S. Sandhu, J. Lewtas, L. Claxton, N. Chernoff, and S. Nesnow (eds.). Plenum Publishing Corp., New York.

Barrett, J. C., T. W. Hesterberg, and D. G. Thomassen. 1984. Use of cell transformation systems for carcinogenicity testing and mechanistic studies of carcinogenesis. Pharmacological Rev. 53S-69S.

Cohen, G. M., A. C. Marchok, P. Nettesheim, V. E. Steele, F. Nelson, S. Huang, and J. K. Selkirk. 1979. Comparative metabolism of benzo(a)pyrene in organ and cell cultures derived from rat tracheas. Cancer Res. 39: 1980-1984.

Federal Register. 1984. Proposed revised national ambient air quality standards for particulate matter. 49: 10408-10430.

Frazier, M. E., and T. K. Andrews. 1983. Transformation of Syrian hamster embryo cells by synfuel mixtures. J. Toxicol. Environ. Health 11: 591-606.

Kendrick, J., P. Nettesheim, and A. S. Hammons. 1974. Tumor induction in tracheal grafts: a new experimental model for respiratory carcinogenesis studies. J. Natl. Cancer Inst. 52: 1317-1325.

Lane, B. P., and S. L. Miller. 1974. Dose-dependence of carcinogen-induced changes in tracheal epithelium. pp. 507-513. E. Karbe and J. F. Parks (eds.).

In: Experimental Lung Cancer Carcinogenesis and Bioassays. Springer-Verlag, New York.

Lang, D. R., H. Kurzepa, M. S. Cole, and J. C. Loper. 1980. Malignant transformation of Balb/3T3 cells by residue organic mixtures from drinking water. J. Environ. Pathol. Toxicol. 4: 41-54.

Li, J. Y., B. Q. Liu, and G. Y. Li, et al. 1979. Atlas of Cancer Mortality in the Peoples Republic of China. China Map Press, Shanghai.

Marchok, A. C., S. F. Huang, and D. H. Martin. 1984. Selection of carcinogen-altered rat tracheal epithelial cells pre-exposed to 7,12-dimethylbenz[a]anthracene by their loss of a need for pyruvate to survive in culture. Carcinogenesis 5: 789-796.

Mass, M. J., and D. G. Kaufman. 1978. [^{3}H]Benzo(a)pyrene metabolism in tracheal epithelial microsomes and tracheal organ cultures. Cancer Res. 38: 3861-3866.

Mass, M. J., and D. G. Kaufman. 1983a. A comparison between the activation of benzo(a)pyrene in organ cultures and microsomes from the tracheal epithelium of rats and hamsters. Carcinogenesis 4: 297-303.

Mass, M. J., and D. G. Kaufman. 1983b. Species differences in the activation of benzo(a)pyrene in the tracheal epithelium of rats and hamsters, pp. 331-351. In: Species and Organ Specificity in Chemical Carcinogenesis. R. Langenbach, S. Nesnow, and J. Rice (eds.). Plenum Publishing Corp. New York.

Mass, M. J., and D. G. Kaufman. 1984. Biochemical studies of the tracheobronchial epithelium. Environ. Health Perspect. 56: 61-74.

Mass, M. J., D. G. Kaufman, J. M. Siegfried, V. E. Steele, and S. Nesnow (eds.). 1985. Cancer of the Respiratory Tract: Predisposing Factors, Raven Press, New York.

Mass, M. J., P. Nettesheim, D. K. Beeman, and J. C. Barrett. 1984a. Inhibition of transformation of primary rat tracheal epithelial cells by retinoic acid. Cancer Res. 44: 5688-5691.

Mass, M. J., P. Nettesheim, T. E. Gray, and J. C. Barrett. 1984b. The effect of 12-O-tetradecanoylphorbol-13-acetate and other tumor promoters on the colony formation of rat tracheal epithelial cells in culture. Carcinogenesis 5: 1597-1601.

Mossman, B. T., and J. E. Craighead. 1974. Topical application of polycyclic hydrocarbons to differentiated respiratory epithelium in long-term organ cultures. pp. 514-520. E. Karbe and J. F. Parks (eds.). In: Experimental Lung Cancer-Carcinogenesis and Bioassays. Springer-Verlag, New York.

Nettesheim, P., and J. C. Barrett. 1984. Tracheal epithelial cell transformation: a model system for studies on neoplastic progression. Crit. Rev. Toxicol. 12: 215-239.

Nishioka, M. G., B. A. Petersen, and J. Lewtas. 1982. Comparison of nitroaromatic content and direct-acting mutagenicity of diesel emissions. pp. 603-612. In: Polynuclear Aromatic Hydrocarbons: Physical and Biological Chemistry. M. Cooke, A. J. Dennis, and G. L. Fisher (eds.). Battelle Press, Columbus.

Schiff, L. J., M. M. Byrne, and J. A. Graham. 1981. Fly ash-induced changes in hamster tracheal epithelum in vivo and in vitro. J. Toxicol. and Environ. Health 8: 431-448.

Steele, V. E., and M. J. Mass. 1985. A rat tracheal cell culture transformation system for assessment of environmental agents as carcinogens and promoters. Environ. Int. 11: 323-329.

Steele, V. E., A. C. Marchok, and P. Nettesheim. 1979. Oncogenic transformation derived from tracheal explants exposed in vitro to N-methyl-N'-nitro-N-nitrosoguanidine. Cancer Res. 39: 3805-3811.

Thomassen, D. G., T. E. Gray, M. J. Mass,, and J. C. Barrett. 1983. High frequency of carcinogen-induced early, preneoplastic changes in rat tracheal epithelial cells in culture Cancer Res. 43: 5956-5963.

Table 1. RTE Cell Transformation by Benzo(a)pyrene

Concentration[a]	CFE ± S.D.	No. of dishes with one or or more transformants[b]	Transformation frequency (%)[c]
0	7.53 ± 1.06	0	0
1	8.48 ± 0.83	3	0.14
2	8.16 ± 0.41	9	0.49
5	8.91 ± 0.49	14	0.87
10	8.94 ± 0.77	12	0.71

[a]in μg/ml.

[b]Dishes were seeded with 2000 RTE cells each; 25 dishes were used for assessing transformation per concentration of BP.

[c]Calculated from the Poisson distribution, by $P_0=e^{-n}$, where P_0 is the fraction of dishes without transformed colonies, n is the average number of transforming events per dish; n/CFC is the transformation frequency per colony-forming cell, where CFC is the number of colony-forming cells per dish.

Table 2. Analysis of Selected PAH in Exhaust Extracts[a]

PAH	Smokey Coal LB(HAi)LKX	Smokeless Coal CG(HBi)YTS	Wood HW(HWi)SSC
Nitropyrenes	N.D.[b]	N.D.	N.D.
Anthracene	734	259	215
1-Methylphenanthrene	791	405	321
Pyrene	1540	1620	654
Benz(a)anthracene	1630	2060	287
1-Methylbenz(a)anthracene	314	281	12
Benzo(a)pyrene	984	1160	204
Benzo(e)pyrene	1340	2910	109
Cyclopenta(cd)pyrene	918	830	387
Benzo(ghi)perylene	1140	161	119

[a]In μg/g of extract.

[b]Not detected.

Table 3. RTE Cell Transformation by Smokey Coal Exhaust Extract LB(HAi)LKX

Experiment	CFE (control)	CFE (treated)	No. of positive dishes[a]	Transformation frequency (%)[b]
1[c]	5.72 ± 0.5	7.68 ± 0.8	9	0.5
2	2.47 ± 0.4	3.48 ± 0.5	7	0.42
3	5.07 ± 0.6	5.33 ± 0.3	8	0.36
4	6.17 ± 0.7	4.61 ± 0.4	9	0.53

[a]Dishes were seeded with 2000 cells each; 25 dishes were used to assess transformation in each experiment; shown is the number of dishes with one or more transformed colony; concentration used was 30 μg/mL.

[b]Calculated from the Poisson distribution, by $P_0=e^{-n}$, where P_0 is the fraction of dishes without transformed colonies, n is the average number of transforming events per dish; n/CFC is the transformation frequency per colony-forming cell, where CFC is the number of colony-forming cells per dish. Results are corrected for spontaneous background.

[c]This experimental sample was derived from another home that used smokey coal as fuel; sample CG(HAi)SSL.

Table 4. RTE Cell Transformation by Smokeless Coal Exhaust Extract CG(HBi)YTS

Experiment	CFE (control)	CFE (treated)	No. of positive dishes[a]	Transformation frequency (%)[b]
1	6.67 ± 0.78	7.71 ± 0.83	4	0.12
2	4.38 ± 0.58	6.79 ± 0.96	13	0.82
3	5.77 ± 0.28	9.11 ± 0.56	4	0.25
4	4.21 ± 0.19	1.42 ± 0.48	3	0.50
5	5.29 ± 0.80	2.52 ± 0.54	11	2.48

[a]Dishes were seeded with 2000 cells each; 25 dishes were used to assess transformation in each experiment; shown is the number of dishes with one or more transformed colony; concentration used was 50 μg/ml.

[b]Calculated from the Poisson distribution, by $P_0=e^{-n}$, where P_0 is the fraction of dishes without transformed colonies, n is the average number of transforming events per dish; n/CFC is the transformation frequency per colony-forming cell, where CFC is the number of colony-forming cells per dish. Results are corrected for spontaneous background.

Table 5. RTE cell transformation by wood exhaust extract HW(HWi)SSC

Experiment	CFE (control)	CFE (treated)	No. of positive dishes[a]	Transformation frequency (%)[b]
1	4.56 ± 0.60	3.81 ± 0.20	8	0.58
2	2.68 ± 0.34	3.38 ± 0.49	13	1.39
3	3.80 ± 0.49	4.50 ± 0.53	3	0.13

[a]Dishes were seeded with 2000 cells each; 25 dishes were used to assess transformation for each experiment; shown is the number of dishes with one or more transformed colony; concentration used was 30 μg/mL.

[b]Calculated from the Poisson distribution, by $P_0=e^{-n}$, where P_0 is the fraction of dishes without transformed colonies, n is the average number of transforming events per dish; n/CFC is the transformation frequency per colony-forming cell, where CFC is the number of colony-forming cells per dish. Results are corrected for spontaneous background.

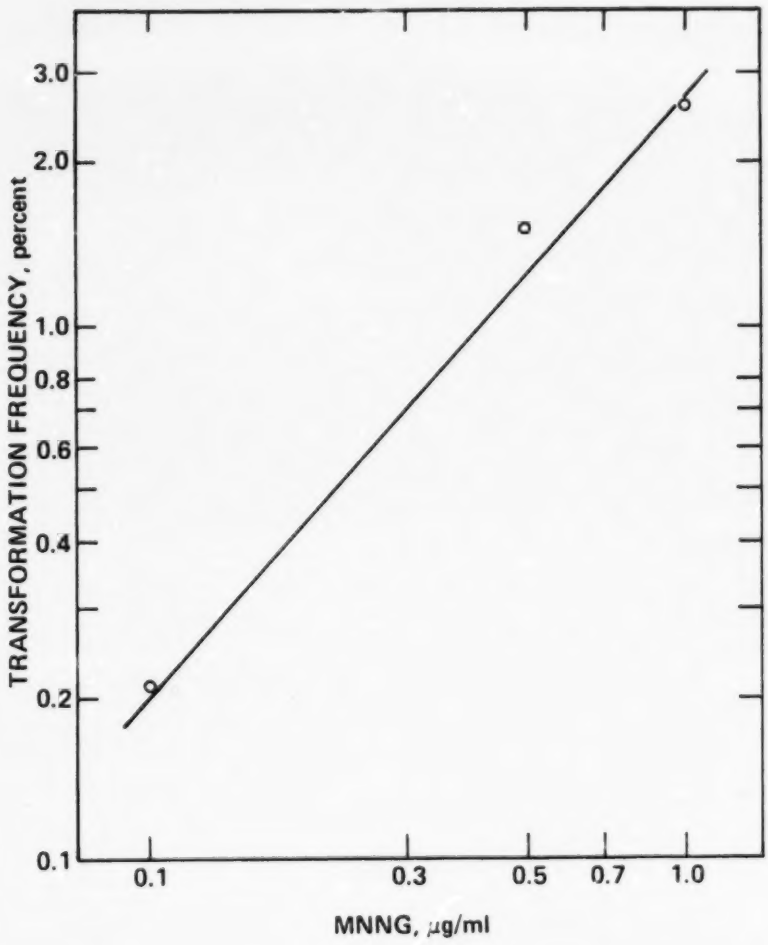

Chart 1. Concentration-response curve for transformation of RTE cells by MNNG. Modified from Thomassen et al., 1983.

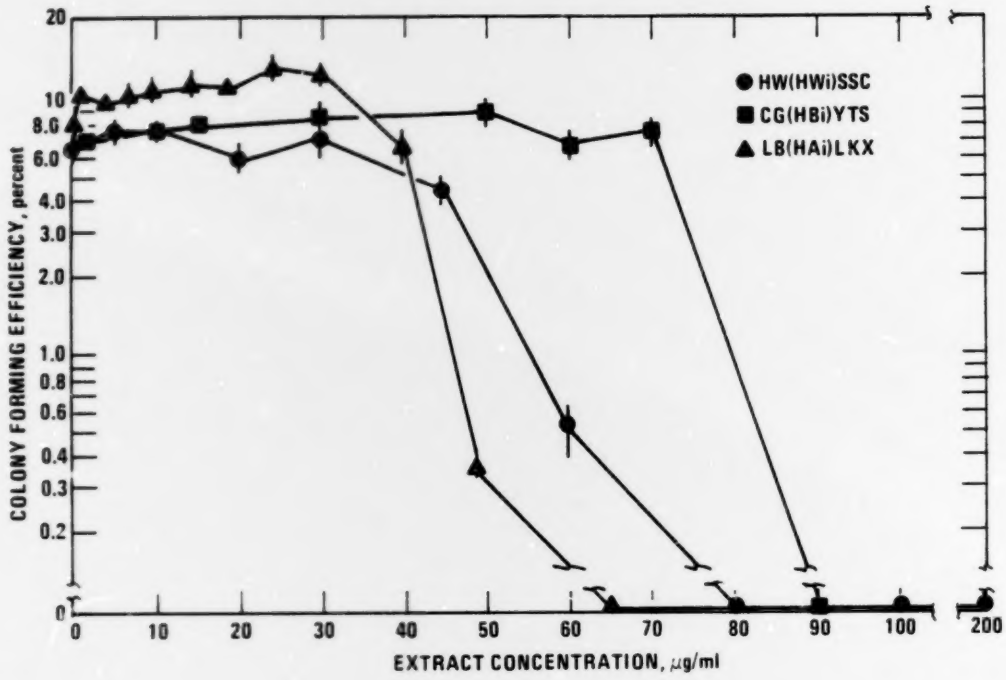

Chart 2. Concentration-response curves for toxicity of environmental effluents: smokey coal, smokeless coal, and wood exhaust extract, tested in RTE cells.